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(54) Title: MAMMALIAN HYALURONAN SYNTHASES, NUCLEIC ACIDS, USES THEREOF		
(57) Abstract <p>The present invention relates to an isolated or recombinant nucleic acid which encodes a mammalian hyaluronan synthase (e.g., human). The present invention also relates to a host cell comprising the nucleic acid encoding mammalian hyaluronan synthase. The present invention also relates to a method for producing a mammalian hyaluronan synthase comprising introducing into a host cell a nucleic acid construct comprising a nucleic acid which encodes a mammalian hyaluronan synthase, whereby a recombinant host cell is produced having said coding sequence operably linked to at least one expression control sequence; and maintaining the host cells produced in a suitable medium under conditions whereby the nucleic acid is expressed. The present invention also relates to an antibody or functional portion thereof which binds mammalian hyaluronan synthase. The present invention also relates to a method of detecting mammalian hyaluronan synthase in a sample comprising contacting a sample with an antibody which binds hyaluronan synthase under conditions suitable for specific binding of said antibody to the mammalian hyaluronan synthase; and detecting antibody-mammalian hyaluronan synthase. The invention further relates to a method of using hyaluronan synthase to make hyaluronan.</p>		

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MAMMALIAN HYALURONAN SYNTHASES, NUCLEIC ACIDS, USES THEREOFBACKGROUND

Hyaluronan is a constituent of the extracellular matrix of connective tissue, and is actively synthesized during wound healing and tissue repair to provide a framework for ingrowth of blood vessels and fibroblasts. Changes in the serum concentration of hyaluronan are associated with inflammatory and degenerative arthropathies such as rheumatoid arthritis. In addition, hyaluronan has been implicated as an important substrate for migration of adhesion of leukocytes during inflammation.

Hyaluronan (hyaluronic acid, HA) is a high molecular mass polysaccharide that has ubiquitous distribution in the extracellular matrix, with highest concentrations in soft connective tissue. It is a linear polysaccharide comprising alternating glucuronic acid and N-acetylglucosamine residues linked by β -1-3 and β -1-4 glycosidic bonds (Laurent, T.C. et al. (1986), "The properties and turnover of hyaluronan." Functions of proteoglycans (Symposium, C.F., Ed. 124, Chichester, England). By interacting with other matrix molecules, such as chondroitin sulfate proteoglycans, hyaluronan provides stability and elasticity to the extracellular matrix. Hyaluronan has several physiochemical and biological functions such as space filling, lubrication, and providing a hydrated matrix through which cells can migrate (Toole, B.P. et al., *Hyaluronate-cell interactions*. The role of the extracellular matrix in development, (Trelstad, R.L., Ed., Alan R. Liss, New York (1984); Laurent, T.C. et al., *Faseb J.* 6:2397-2404 (1992)). Interaction of hyaluronan with the leukocyte cell surface receptor CD44 has been

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shown to contribute to organ specific leukocyte homing and migration (Jalkanen, S.T. et al., *J. Cell. Biol.*, 105:893-990 (1987); Aruffo, A., et al., *Cell* 61:1303-1313 (1990); Culty, M. et al., *J. Cell. Biol.*, 111:2765-2774 (1990); Miyake, K. et al., *J. Exp. Med.* 172:69-75 (1990); Sherman, L. et al., *Current Opinions in Cell Biology*, 6:726-733 (1994)). Hyaluronan synthesis has been suggested to be required for cellular proliferation (Brecht, M. et al., *Biochem. J.* 239:445-450 (1986); Hronowski, L. and Anastassiades, T.P., *J. Biol. Chem.* 255:9210-9217 (1980); Matuoka, K. et al., *J. Cell Biol.* 104:1105-1115 (1987); Mian, N., *Biochem. J.* 237:333-342 (1986); Tomida, M. et al., *J. Cell Physiol.* 86:121-130 (1975)), and over-expression of receptors for hyaluronan, including a receptor for hyaluronan mediated motility (RHAMM) and CD44, correlates with increased levels of tumor metastasis (Gunthert, U., *Curr. Topics Microbiol. Immunol.* 184:47-63 (1993); Hall, C.L. et al., *Cell* 82:19-28 (1995); Turley, E.A., *Cancer and Metastasis Reviews* 11:1233-1241 (1992)). Purified preparations of hyaluronan exhibit unique viscoelastic properties, and as a consequence of these characteristics have been used in viscoelastic surgery and viscosupplementation (Balazs, E.A., and Denninger, J.L., *Clinical uses of hyaluronan, The biology of hyaluronan, Ciba foundation symposium*, Wiley, Chichester, England (1989)). Hyaluronan is synthesized mainly by mesenchymal cells and the accumulation of HA is an early event in tissue repair. The serum level of hyaluronan is elevated in inflammatory settings such as rheumatoid arthritis, osteoarthritis, liver cirrhosis, Werner's syndrome, renal failure and psoriasis (Laurent, T.C. et al., *Faseb J.* 6:2397-2404 (1992); Laurent, T.C. *Annals of Medicine* 28:in press (1996)).

Hyaluronan is synthesized by a membrane bound synthase; monosaccharide and disaccharide residues are

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added to the reducing end of the polysaccharide as it protrudes through the plasma membrane (Prehm, P., *Biochem. J.* 211:181-189 (1983); Prehm, P., *Biochem. J.* 220:597-600 (1984)). Regulation of hyaluronan biosynthesis has been

5 studied in several tissue culture systems. Factors involved in tissue growth and repair such as different isoforms of platelet derived growth factor (PDGF-AA, PDGF-BB), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and transforming growth factor β (TGF- β), all

10 exhibit stimulatory activity on hyaluronan biosynthesis (Heldin, P. et al., *Biochem. J.* 258, 919-922 (1992)).

A cDNA encoding a bacterial hyaluronan synthase has been cloned from *Streptococcus pyogenes* (*hasA*) (DeAngelis, J.P. et al., *J. Biol. Chem.* 268, 19181-19184 (1993)).

15 Other related genes with N-acetylglucosaminyl transferase activity have been isolated from the nitrogen fixing bacteria *Rhizobium* (*nodC*) and chitin synthases (Chs) from *Saccharomyces* (DeAngelis, P.L. et al., *Biochem. Biophys. Res. Comm.* 199:1-10 (1994)). A putative vertebrate

20 homolog, (DG42), was cloned from *Xenopus laevis* and has also been speculated to be a glycosaminoglycan synthetase (Rosa, F. et al., *Develop. Biol.* 129:114-123 (1988)). To date, however, a mammalian hyaluronan synthase gene has not been identified.

25 SUMMARY OF THE INVENTION

The present invention relates to isolated and/or recombinant nucleic acids which encode a mammalian hyaluronan synthase (e.g., human). In one embodiment, the nucleic acid of the present invention comprises SEQ ID

30 NO:1. In another embodiment, the invention relates to a nucleic acid wherein said nucleic acid hybridizes under stringent conditions with a second nucleic acid having a nucleotide sequence of SEQ ID NO: 1.

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The present invention also relates to a host cell comprising a nucleic acid encoding mammalian hyaluronan synthase. In a particular embodiment, the host cell comprises nucleic acid encoding mammalian hyaluronan
5 synthase which is operably linked to an expression control sequence, whereby mammalian hyaluronan synthase is expressed when the host cell is maintained under conditions suitable for expression.

The present invention also relates to a method for
10 producing a mammalian hyaluronan synthase comprising introducing into a host cell a nucleic acid construct comprising a nucleic acid which encodes a mammalian hyaluronan synthase, whereby a recombinant host cell is produced having said coding sequence operably linked to an
15 (i.e., at least one) expression control sequence; and maintaining the host cells produced in a suitable medium under conditions whereby the nucleic acid is expressed.

The present invention also relates to an antibody or functional portion thereof (e.g., an antigen binding
20 portion such as an Fv, Fab, Fab', or F(ab')₂ fragment) which binds mammalian hyaluronan synthase.

The present invention also relates to a method of detecting mammalian hyaluronan synthase in a sample comprising contacting a sample with an antibody which binds
25 hyaluronan synthase under conditions suitable for specific binding of said antibody to the mammalian hyaluronan synthase; and detecting antibody-mammalian hyaluronan synthase.

The invention further relates to a method of using
30 hyaluronan synthase to make hyaluronan.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A is a graph illustrating that CHO cells transfected with human hyaluronan synthase cDNA synthesize hyaluronic acid; media and cell lysates were combined and

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then incubated overnight in the absence (o---o) or presence (•---•) of 10U Streptomyces hyaluronidase/ml and subjected to chromatography on Sephadex G-50 columns; Streptomyces hyaluronidase-sensitive radioactivity represents

5 synthesized hyaluronan.

Figure 1B is a graph illustrating that CHO cells not transfected with human hyaluronan synthase cDNA produce very little high molecular weight streptomyces hyaluronidase-sensitive material.

10 Figure 2 is an illustration of the nucleotide sequence (SEQ ID NO:1) and deduced protein sequence (SEQ ID NO:2) determined from human hyaluronan synthase cDNA clone 30C; cysteine residues are circled and a conserved motif, B(X₇)B, believed to be important for binding hyaluronan is
15 lightly outlined; consensus phosphorylation sequences for protein kinase C (RHLT, KYT and RWLS) and cAMP dependent protein kinases (RWS) are outlined in bold; also shown with a bold underline at position 2066 is a consensus polyadenylation signal, AATAAA. (Standard single letter
20 amino acid codes are used.)

Figure 3A is an amino acid alignment of the human hyaluronan synthase protein sequence (SEQ ID NO:2) with the DG42 sequence from *Xenopus laevis* (SEQ ID NO:3) and hasA sequence of *Streptococcus pyogenes* (SEQ ID NO:4) prepared
25 using the DNASTar program and the Clustal method with default parameters for gap penalties.

Figure 3B is a comparison of Kyte-Doolittle hydrophilicity profiles of human hyaluronan synthase, DG42 and hasA.

30 Figure 3C is a proposed structure of human hyaluronan synthase, indicating approximate boundaries of transmembrane regions and intra- and extracellular loops; a hyaluronan binding motif (HBM), B(X₇)B, is indicated at the amino portion of a large predicted intracellular loop;
35 approximate locations of protein kinase C consensus sites

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are indicated by open circles, while a single cAMP dependent kinase site is shown as a filled circle.

Figure 4A is a Northern blot probed with the full length insert of the human hyaluronan synthase cDNA clone 30C; the blot was subsequently stripped and reprobbed with a β -actin cDNA as a control.

Figure 4B is a Southern blot initially hybridized with full-length human hyaluronan synthase cDNA, washed at 50°C, and exposed overnight; a considerable amount of background was seen although specific bands could be detected; subsequently the blot was stripped and probed with a 450 bp Sac II fragment encompassing the 3' end of the cDNA; this probe gave a similar pattern with less background (likely due to a lower GC content).

15 DETAILED DESCRIPTION OF THE INVENTION

Proteins and Peptides

The present invention relates to isolated and/or recombinant (including, e.g., essentially pure) proteins or polypeptides designated mammalian hyaluronan synthase and variants of mammalian hyaluronan synthase. In a preferred embodiment, the isolated and/or recombinant proteins of the present invention have at least one property, activity or function characteristic of a mammalian hyaluronan synthase (as defined herein), such as activity in the synthesis of hyaluronan and/or ability to confer cell adhesion by the lymphocyte receptor CD44 (i.e., human CD44 or a mammalian homolog thereof).

Proteins or polypeptides referred to herein as "isolated" are proteins or polypeptides purified to a state beyond that in which they exist in mammalian cells.

"Isolated" proteins or polypeptides include proteins or polypeptides obtained by methods described herein, similar methods or other suitable methods, including essentially pure proteins or polypeptides, proteins or polypeptides

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produced by chemical synthesis (e.g., synthetic peptides), or by combinations of biological and chemical methods, and recombinant proteins or polypeptides which are isolated. The proteins can be obtained in an isolated state of at least about 50 % by weight, preferably at least about 75 % by weight, and more preferably, in essentially pure form. Proteins or polypeptides referred to herein as "recombinant" are proteins or polypeptides produced by the expression of recombinant nucleic acids.

10 As used herein "mammalian hyaluronan synthase" refers to naturally occurring or endogenous mammalian hyaluronan synthase proteins, to proteins having an amino acid sequence which is the same as that of a naturally occurring or endogenous corresponding mammalian hyaluronan synthase (e.g., recombinant proteins), and to functional variants of each of the foregoing (e.g., functional fragments and/or mutants produced via mutagenesis and/or recombinant techniques). Accordingly, as defined herein, the term includes mature mammalian hyaluronan synthase, glycosylated or unglycosylated mammalian hyaluronan synthase proteins, polymorphic or allelic variants, and other isoforms of mammalian hyaluronan synthase (e.g., produced by alternative splicing or other cellular processes), and functional fragments.

25 Naturally occurring or endogenous mammalian hyaluronan synthase proteins include wild type proteins such as mature mammalian hyaluronan synthase, polymorphic or allelic variants and other isoforms which occur naturally in mammals (e.g., primate, preferably human, murine, bovine). Such proteins can be recovered from a source which naturally produces mammalian hyaluronan synthase, for example. These mammalian proteins having the same amino acid sequence as naturally occurring or endogenous corresponding mammalian hyaluronan synthase, are referred to by the name of the corresponding mammal. For example,

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as described herein, where the corresponding mammal is human, the protein is designated as a human hyaluronan synthase (HAS), such as recombinant human hyaluronan synthase produced in a suitable host cell.

5 "Functional variants" of mammalian hyaluronan synthase include functional fragments, functional mutant proteins, and/or functional fusion proteins. Generally, fragments or portions of mammalian hyaluronan synthase encompassed by the present invention include those having a deletion
10 (i.e., one or more deletions) of an amino acid (i.e., one or more amino acids) relative to the mature mammalian hyaluronan synthase (such as N-terminal, C-terminal or internal deletions). Fragments or portions in which only contiguous amino acids have been deleted or in which
15 non-contiguous amino acids have been deleted relative to mature mammalian hyaluronan synthase are also envisioned.

Generally, mutants or derivatives of mammalian hyaluronan synthase, encompassed by the present invention include natural or artificial variants differing by the
20 addition, deletion and/or substitution of one or more contiguous or non-contiguous amino acid residues, or modified polypeptides in which one or more residues is modified, and mutants comprising one or more modified residues. Preferred mutants are natural or artificial
25 variants of mammalian hyaluronan synthase differing by the addition, deletion and/or substitution of one or more contiguous or non-contiguous amino acid residues.

A "functional fragment or portion", "functional mutant" and/or "functional fusion protein" of a mammalian
30 hyaluronan synthase refers to an isolated and/or recombinant protein or oligopeptide which has at least one property, activity and/or function characteristic of a mammalian hyaluronan synthase, such as activity or function characteristic of a mammalian hyaluronan synthase (as
35 defined herein), such as activity in the synthesis of

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hyaluronan and/or ability to confer cell adhesion by the lymphocyte receptor CD44.

Suitable fragments or mutants can be identified by screening. For example, the N-terminal, C-terminal, or
5 internal regions of the protein can be deleted in a step-wise fashion and the resulting protein or polypeptide can be screened using a suitable binding or adhesion assay. Where the resulting protein displays activity in the assay, the resulting protein ("fragment") is functional.
10 Information regarding the structure and function of other hyaluronan synthases (e.g., hasA, DG42), and of HAS as shown herein, provides a basis for dividing HAS into functional domains.

The term variant also encompasses fusion proteins,
15 comprising a mammalian hyaluronan synthase (e.g., mature mammalian hyaluronan synthase) as a first moiety, linked to a second moiety not occurring in the mammalian hyaluronan synthases found in nature. Thus, the second moiety can be an amino acid, oligopeptide or polypeptide. The first
20 moiety can be in an N-terminal location, C-terminal location or internal to the fusion protein. In one embodiment, the fusion protein comprises a mammalian hyaluronan synthase or portion thereof as the first moiety, and a second moiety comprising a linker sequence and
25 affinity ligand (e.g., an enzyme, an antigen, epitope tag).

Examples of "mammalian hyaluronan synthase" proteins include proteins having an amino acid sequence as set forth or substantially as set forth in Figure 2 (SEQ ID NO:2) and functional portions thereof. In a preferred embodiment, a
30 mammalian hyaluronan synthase or variant has an amino acid sequence which has at least about 50% identity, more preferably at least about 75% identity, and still more preferably at least about 90% identity, to the protein shown in Figure 2 (SEQ ID NO:2).

Method of Producing Recombinant Proteins

Another aspect of the invention relates to a method of producing a mammalian hyaluronan synthase or variant (e.g., portion) thereof. Recombinant protein can be obtained, for example, by the expression of a recombinant DNA molecule encoding a mammalian hyaluronan synthase or variant thereof in a suitable host cell, for example.

Constructs suitable for the expression of a mammalian hyaluronan synthase or variant thereof are also provided. The constructs can be introduced into a suitable host cell, and cells which express a recombinant mammalian hyaluronan synthase or variant thereof, can be produced and maintained in culture. Such cells are useful for a variety of purposes, and can be used in the production of protein for characterization, isolation and/or purification, (e.g., affinity purification), and as immunogens, for instance. Suitable host cells can be procaryotic, including bacterial cells such as *E. coli*, *B. subtilis* and or other suitable bacteria (e.g., *Streptococci*) or eucaryotic, such as fungal or yeast cells (e.g., *Pichia pastoris*, *Aspergillus species*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Neurospora crassa*), or other lower eucaryotic cells, and cells of higher eucaryotes such as those from insects (e.g., Sf9 insect cells) or mammals (e.g., Chinese hamster ovary cells (CHO), COS cells, HuT 78 cells, 293 cells). (See, e.g., Ausubel, F.M. et al., eds. *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons Inc., (1993)).

Host cells which produce a recombinant mammalian hyaluronan synthase or variants thereof can be produced as follows. For example, a nucleic acid encoding all or part of the coding sequence for the desired protein can be inserted into a nucleic acid vector, e.g., a DNA vector, such as a plasmid, virus or other suitable replicon for expression. A variety of vectors are available, including

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vectors which are maintained in single copy or multiple copy, or which become integrated into the host cell chromosome.

The transcriptional and/or translational signals of a mammalian hyaluronan synthase gene can be used to direct expression. Alternatively, suitable expression vectors for the expression of a nucleic acid encoding all or part of the coding sequence of the desired protein are available. Suitable expression vectors can contain a number of components, including, but not limited to one or more of the following: an origin of replication; a selectable marker gene; one or more expression control elements, such as a transcriptional control element (e.g., a promoter, an enhancer, terminator), and/or one or more translation signals; a signal sequence or leader sequence for membrane targeting or secretion (of mammalian origin or from a heterologous mammal or non-mammalian species). In a construct, a signal sequence can be provided by the vector, the mammalian hyaluronan synthase coding sequence, or other source.

A promoter can be provided for expression in a suitable host cell. Promoters can be constitutive or inducible. The promoter is operably linked to a nucleic acid encoding the mammalian hyaluronan synthase or variant thereof, and is capable of directing expression of the encoded polypeptide in the host cell. A variety of suitable promoters for procaryotic (e.g., lac, tac, T3, T7 promoters for *E. coli*) and eucaryotic (e.g., yeast alcohol dehydrogenase (ADH1), SV40, CMV) hosts are available.

In addition, the expression vectors typically comprise a selectable marker for selection of host cells carrying the vector, and in the case of a replicable expression vector, an origin of replication. Genes encoding products which confer antibiotic or drug resistance are common selectable markers and may be used in procaryotic (e.g., β -

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lactamase gene (ampicillin resistance), *Tet* gene for tetracycline resistance) and eucaryotic cells (e.g., neomycin (G418 or geneticin), gpt (mycophenolic acid), ampicillin, or hygromycin resistance genes). Dihydrofolate
5 reductase marker genes permit selection with methotrexate in a variety of hosts. Genes encoding the gene product of auxotrophic markers of the host (e.g., *LEU2*, *URA3*, *HIS3*) are often used as selectable markers in yeast. Use of viral (e.g., baculovirus) or phage vectors, and vectors
10 which are capable of integrating into the genome of the host cell, such as retroviral vectors, are also contemplated. The present invention also relates to cells carrying these expression vectors.

For example, a nucleic acid encoding a mammalian
15 hyaluronan synthase or variant thereof can be incorporated into a vector, operably linked to one or more expression control elements, and the construct can be introduced into host cells which are maintained under conditions suitable for expression, whereby the encoded polypeptide is
20 produced. The construct can be introduced into cells by a method appropriate to the host cell selected (e.g., transformation, transfection, electroporation, infection). For production of a protein, host cells comprising the construct are maintained under conditions appropriate for
25 expression, (e.g., in the presence of inducer, suitable media supplemented with appropriate salts, growth factors, antibiotic, nutritional supplements, etc.). The encoded protein (e.g., human hyaluronan synthase) can be isolated from the host cells or medium.

30 Fusion proteins can also be produced in this manner. For example, some embodiments can be produced by the insertion of a mammalian hyaluronan synthase cDNA or portion thereof into a suitable expression vector, such as Bluescript®II SK +/- (Stratagene), pGEX-4T-2 (Pharmacia),
35 pcDNA-3 (Invitrogen) and pET-15b (Novagen). The resulting

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construct can then be introduced into a suitable host cell for expression. Upon expression, fusion protein can be isolated or purified from a cell lysate by means of a suitable affinity matrix (see e.g., *Current Protocols in Molecular Biology* (Ausubel, F.M. et al., eds., Vol. 2, Suppl. 26, pp. 16.4.1-16.7.8 (1991))). In addition, affinity labels provide a means of detecting a fusion protein. For example, the cell surface expression or presence in a particular cell fraction of a fusion protein comprising an antigen or epitope affinity label can be detected by means of an appropriate antibody.

Nucleic Acids, Constructs and Vectors

The present invention relates to isolated and/or recombinant (including, e.g., essentially pure) nucleic acids (e.g., polynucleotides) having sequences which encode a mammalian hyaluronan synthase or variant thereof as described herein.

Nucleic acids referred to herein as "isolated" are nucleic acids separated away from the nucleic acids of the genomic DNA or cellular RNA of their source of origin (e.g., as it exists in cells or in a mixture of nucleic acids such as a library), and may have undergone further processing. "Isolated" nucleic acids include nucleic acids obtained by methods described herein, similar methods or other suitable methods, including essentially pure nucleic acids, nucleic acids produced by chemical synthesis, by combinations of biological and chemical methods, and recombinant nucleic acids which are isolated (see e.g., Daugherty, B.L. et al., *Nucleic Acids Res.*, 19(9):2471-2476 (1991); Lewis, A.P. and J.S. Crowe, *Gene*, 101: 297-302 (1991)). Nucleic acids referred to herein as "recombinant" are nucleic acids which have been produced by recombinant DNA methodology, including those nucleic acids that are generated by procedures which rely upon a method of

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artificial recombination, such as the polymerase chain reaction (PCR) and/or cloning into a vector using restriction enzymes. "Recombinant" nucleic acids are also those that result from recombination events that occur through the natural mechanisms of cells, but are selected for after the introduction to the cells of nucleic acids designed to allow and make probable a desired recombination event.

In one embodiment, the nucleic acid or portion thereof encodes a protein or polypeptide having at least one property, activity or function characteristic of a mammalian hyaluronan synthase (as defined herein), such as activity or function characteristic of a mammalian hyaluronan synthase (as defined herein), such as activity in the synthesis of hyaluronan and/or ability to mediate cell adhesion by the lymphocyte receptor CD44.

The present invention also relates more specifically to isolated and/or recombinant nucleic acids or a portion thereof having sequences which encode mammalian hyaluronan synthase or variants thereof.

The invention relates to isolated and/or recombinant nucleic acids that are characterized by:

(1) their ability to hybridize to (a) a nucleic acid encoding a mammalian hyaluronan synthase, such as a nucleic acid having a nucleotide sequence as set forth or substantially as set forth in Figure 2 (SEQ ID NO:1); (b) the complement of (a); or (c) portions of either of the foregoing (e.g., a portion comprising the open reading frame); or

(2) by their ability to encode a polypeptide having the amino acid sequence of a mammalian hyaluronan synthase (e.g., SEQ ID NO:2); or

(3) by both characteristics.

In one embodiment, the nucleic acid shares at least about 50% nucleotide sequence similarity to the nucleotide

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sequences shown in Figure 2 (SEQ ID NO:1). More preferably, the nucleic acid shares at least about 75% nucleotide sequence similarity, and still more preferably, at least about 90% nucleotide sequence similarity, to the sequence shown in Figure 2 (SEQ ID NO:1).

Isolated and/or recombinant nucleic acids meeting these criteria comprise nucleic acids having sequences identical to sequences of naturally occurring mammalian hyaluronan synthase or variants of the naturally occurring sequences. Such variants include mutants differing by the addition, deletion or substitution of one or more residues, modified nucleic acids in which one or more residues are modified (e.g., DNA or RNA analogs), and mutants comprising one or more modified residues.

A nucleic acid of the present invention may be in the form of RNA or in the form of DNA (e.g., cDNA, genomic DNA, and synthetic DNA). The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptide may be identical to the coding sequence shown in Figure 2 (SEQ ID NO:1) or that of the cDNA in clone 30C or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same, mature polypeptides as the DNA of Figure 2 (SEQ ID NO:2) or the cDNA in clone 30C.

The polynucleotide which encodes a mature polypeptide encoded by the cDNA of clone 30C may include: only the coding sequence of a mature polypeptide; the coding sequence for a mature polypeptide and additional coding sequence such as a leader or secretory sequence; the coding sequence for a mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence.

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Nucleic acids of the present invention, including those which hybridize to a selected nucleic acid as described above, can be detected or isolated under high stringency conditions or moderate stringency conditions, for example. "High stringency conditions" and "moderate stringency conditions" for nucleic acid hybridizations are explained at pages 2.10.1-2.10.16 (see particularly 2.10.8-11) and pages 6.3.1-6 in *Current Protocols in Molecular Biology* (Ausubel, F.M. et al., eds., Vol. 1, Suppl. 26, 1991) the teachings of which are hereby incorporated by reference. Factors such as probe length, base composition, percent mismatch between the hybridizing sequences, temperature and ionic strength influence the stability of nucleic acid hybrids. Thus, high or moderate stringency conditions can be determined empirically, and depend in part upon the characteristics of the known nucleic acid (e.g., DNA) and the other nucleic acids to be assessed for hybridization thereto.

Isolated and/or recombinant nucleic acids that are characterized by their ability to hybridize (e.g., under high or moderate stringency conditions) to (a) a nucleic acid encoding a mammalian hyaluronan synthase (for example, the nucleic acid depicted in Figure 2 (SEQ ID NO:1); (b) the complement of the nucleic acids of (a), (c) or a portion thereof, can also encode a protein or polypeptide having at least one property, activity or function characteristic of a mammalian hyaluronan synthase (as defined herein), such as activity in the synthesis of hyaluronan and/or ability to mediate cell adhesion by the lymphocyte receptor CD44, and in a preferred embodiment encode polypeptides which retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNA of Figure 2 (SEQ ID NO:1) or the cDNA of clone 30C.

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Nucleic acids of the present invention can be used in the production of proteins or polypeptides. For example, a nucleic acid (e.g., DNA) encoding a mammalian hyaluronan synthase can be incorporated into various constructs and
5 vectors created for further manipulation of sequences or for production of the encoded polypeptide in suitable host cells as described above.

A further embodiment of the invention is antisense nucleic acid, which is complementary, in whole or in part,
10 to a target molecule comprising a sense strand, and can hybridize with the target molecule. The target can be DNA, or its RNA counterpart (i.e., wherein T residues of the DNA are U residues in the RNA counterpart). When introduced into a cell, antisense nucleic acid can inhibit the
15 expression of the gene encoded by the sense strand. Antisense nucleic acids can be produced by standard techniques.

In a particular embodiment, the antisense nucleic acid is wholly or partially complementary to and can hybridize
20 with a target nucleic acid, wherein the target nucleic acid can hybridize to a nucleic acid having the sequence of the complement of the strand shown in Figure 2 (SEQ ID NO:1). For example, antisense nucleic acid can be complementary to a target nucleic acid having the sequence shown as the open
25 reading frame in Figure 2 (SEQ ID NO:1) or to a portion thereof sufficient to allow hybridization. In another embodiment, the antisense nucleic acid is wholly or partially complementary to and can hybridize with a target nucleic acid which encodes a mammalian hyaluronan synthase.

30 The nucleic acids can also be used as probes (e.g., in *in situ* hybridization) to assess associations between inflammatory settings (e.g., rheumatoid arthritis, osteoarthritis, liver cirrhosis, Werner's syndrome, renal failure and psoriasis) and increased expression of
35 mammalian hyaluronan synthase in affected tissues or serum.

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The nucleic acids can also be used as probes to detect and/or isolate (e.g., by hybridization with RNA or DNA) polymorphic or allelic variants, for example, in a sample (e.g., inflamed tissue) obtained from a host (e.g. mammalian). Moreover, the presence or frequency of a particular variant in a sample(s) obtained from one or more affected hosts, as compared with a sample(s) from normal host(s), can be indicative of an association between an inflammatory setting and a particular variant, which in turn can be used in the diagnosis of the condition.

As described in the exemplification, functional expression cloning was used to identify a cDNA encoding human hyaluronan synthase, and it was demonstrated that this gene can confer activity both in the synthesis of hyaluronan and as a mediator of cell adhesion by the lymphocyte receptor CD44. A human hyaluronan synthase (HAS) cDNA was isolated by a functional expression cloning approach. Transfection of CHO cells conferred hyaluronidase sensitive adhesiveness of a mucosal T cell line via the lymphocyte hyaluronan receptor, CD44, as well as increased hyaluronan levels in the cultures of transfected cells. The HAS amino acid sequence shows homology to the hasA gene product of *Streptococcus pyogenes* and a putative glycosaminoglycan synthetase from *xenopus laevis*. Expression of HAS message parallels tissues where high levels of hyaluronan synthesis occur, indicating that transcription of synthase mRNA is a critical component of hyaluronate synthesis.

UTILITIES

Mammalian hyaluronan synthases of the present invention can be used to produce hyaluronan. Hyaluronan has a variety of uses, including use in cosmetics and pharmaceuticals (see e.g., EPO, 443,043 B1 and U.S. 5,015,577 the teachings of which are each incorporated

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herein by reference). Hyaluronan or pharmaceutical compositions comprising hyaluronan are useful for treating wounds or surgical incisions and can reduce or prevent hypertrophic scars and keloid formation, and in eye surgery
5 as a replacement for vitreous fluid, for example.

For example, a mammalian hyaluronan synthase or functional variant thereof can be expressed in a suitable host cell under conditions appropriate for production of hyaluronan to occur (e.g., in suitable medium comprising
10 any required precursors). Isolated or purified hyaluronan synthase can also be used to prepare hyaluronan from precursors (e.g., UDP-glucuronic acid and UDP-N-acetylglucosamine).

The present invention also provides antibodies which
15 (1) can bind a "mammalian hyaluronan synthase" *in vitro* and/or *in vivo*; and/or (2) can inhibit an activity or function characteristic of a "mammalian hyaluronan synthase", such as hyaluronan synthesis. Preferably the antibodies are capable of selective binding of mammalian
20 hyaluronan synthase *in vitro* and/or *in vivo* (e.g., bind selectively to mammalian hyaluronan synthase expressed in ovary and/or spleen, thymus, prostate, etc. (e.g., as assessed immunohistologically)).

Preferably, the antibodies can bind a mammalian (e.g.
25 human) hyaluronan synthase with high affinity (for example, a K_a in the range of about 1 - 10 nM, or a K_d in the range of about 1×10^{-8} to 1×10^{-10} mol⁻¹).

The antibodies of the present invention are useful in a variety of applications, including processes, research,
30 diagnostic and therapeutic applications. For instance, they can be used to isolate and/or purify mammalian hyaluronan synthase or variants thereof (e.g., by affinity purification or other suitable methods), and to study mammalian hyaluronan synthase structure (e.g.,
35 conformation) and function.

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The antibodies of the present invention can also be used to modulate mammalian hyaluronan synthase function in diagnostic (e.g., in vitro) or therapeutic applications. For instance, antibodies can act as inhibitors of (reduce
5 or prevent) hyaluronan synthesis, thereby inhibiting process mediated by hyaluronan such as cell adhesion and metastasis.

In addition, antibodies of the present invention can be used to detect and/or measure the level of a mammalian
10 hyaluronan synthase in a sample (e.g., tissues or body fluids, such as an inflammatory exudate, blood, serum, bowel fluid, or on cells transfected with a nucleic acid of the present invention). For example, a sample (e.g., tissue and/or fluid) can be obtained from a host (e.g.,
15 mammalian) and a suitable immunological method can be used to detect and/or measure mammalian hyaluronan synthase levels, including methods such as enzyme-linked immunosorbent assays (ELISA), including chemiluminescence assays, radioimmunoassay, and immunohistology. In one
20 embodiment, a method of detecting a selected mammalian hyaluronan synthase in a sample is provided, comprising contacting a sample with an antibody which binds an isolated mammalian hyaluronan synthase under conditions suitable for specific binding of said antibody to the
25 selected mammalian hyaluronan synthase, and detecting antibody-mammalian hyaluronan synthase complexes which are formed.

In an application of the method, antibodies reactive with a mammalian hyaluronan synthase can be used to analyze
30 normal versus inflamed tissues in mammals for mammalian hyaluronan synthase reactivity and/or expression (e.g., immunohistologically). Thus, the antibodies of the present invention permit immunological methods of assessment of expression of primate (e.g., human mammalian hyaluronan
35 synthase) in normal versus inflamed tissues, through which

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the presence of disease, disease progress and/or the efficacy of anti-mammalian hyaluronan synthase therapy in inflammatory disease can be assessed.

An antibody can be administered in an effective amount which inhibits mammalian hyaluronan synthase activity. For therapy, an effective amount will be sufficient to achieve the desired therapeutic and/or prophylactic effect (such as an amount sufficient to reduce or prevent mammalian hyaluronan synthase-mediated hyaluronan synthesis). The antibody can be administered in a single dose or multiple doses. The dosage can be determined by methods known in the art and is dependent, for example, upon the individual's age, sensitivity, tolerance and overall well-being. Suitable dosages for antibodies can be from 0.1-1.0 mg/kg body weight per treatment.

According to the method, an antibody can be administered to an individual (e.g., a human) alone or in conjunction with another agent (administered before, along with or subsequent to administration of the additional agent).

A variety of routes of administration are possible including, but not necessarily limited to parenteral (e.g., intravenous, intraarterial, intramuscular, subcutaneous injection), oral (e.g., dietary), topical, inhalation (e.g., intrabronchial, intranasal or oral inhalation, intranasal drops), or rectal, depending on the disease or condition to be treated. Parenteral administration is a preferred mode of administration.

Formulation will vary according to the route of administration selected (e.g., solution, emulsion, capsule). An appropriate composition comprising the antibody to be administered can be prepared in a physiologically acceptable vehicle or carrier. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions

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or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles can include
5 various additives, preservatives, or fluid, nutrient or electrolyte replenishers (See, generally, Remington's Pharmaceutical Science, 16th Edition, Mack, Ed. 1980). For inhalation, the compound can be solubilized and loaded into a suitable dispenser for administration (e.g., an atomizer,
10 nebulizer or pressurized aerosol dispenser).

EXEMPLIFICATION

Plasmids, monoclonal antibodies and cell lines

The following plasmids were used as controls in expression cloning and for functional adhesion assays:
15 pSV-SPORT-1 (GIBCO, Gaithersburg, MD) or pCDNA3 (Invitrogen, San Diego, CA) controls and murine MAdCAM-1 in pCDM8 (pCDMAD-7 (Briskin, M.J., *Nature* 363:461-464 (1993))). Monoclonal antibodies used were anti-murine CD-44 TJB1.7 (a gift from T. Yoshino and E. Butcher, Stanford, CA); anti-
20 murine MAdCAM-1 MECA-367 (Streeter, P.R. et al., *Nature* 331:41-46 (1988)); anti-human VCAM-1 2G7 (Graber, N. J. *Immunol.* (145):819 (1990)); anti-murine β 7 FIB 504 (Andrew, D.P. et al., *J. Immunol.* 153:3847-3861 (1994)); and anti-murine α 4 PS/2 (Miyake, K. J. *Exp. Med.* 173:599-607
25 (1991)). Cell lines used for expression cloning and functional adhesion assays were: CHO/P (Heffernan, M. and Dennis, J.D. *Nucl. Acids Res.* 19:85 (1991)) and the murine T cell lymphoma TK1 (Butcher, E.C. et al., *Eur. J. Immunol.* 10:556-561 (1980)).

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cdNA synthesis and library construction

mRNA was isolated from human lymph nodes using standard procedures previously described (Briskin, M.J., *Nature* 363:461-464 (1993)). cdNA was synthesized using the
5 Superscript™ lambda system in conjunction with the pSV-SPORT-1 vector (Gibco, Gaithersburg, MD) essentially using the manufacturer's protocol. The highest molecular weight fractions (>1.5kb) of cdNA were ligated into the pSV-SPORT-1 vector and plated in pools at a density of 5,000
10 clones/plate on 100 LB agar plates with ampicillin (50µg/ml). After incubation overnight, plasmid DNAs were purified from each plate individually by use of QIAprep spin columns (QIAGEN, Chatsworth, CA) according to manufacturer's instructions.

15 Expression cloning

CHO/P cells were seeded into 24 well plates approximately 24 hours prior to transfection at a density of 40,000 cells/well. DNAs were transiently transfected using the LipofectAMINE™ reagent (GIBCO, Gaithersburg, MD)
20 as recently described (Shyjan, A.M. et al., *J. Immunol.*, 156:2851-2857 (1996)).

For the adhesion assays in the expression cloning screen, TK1 cells were resuspended at a density of 2×10^6 /ml in a cell binding assay buffer previously described
25 (Shyjan, A.M. et al., *J. Immunol.*, 156:2851-2857 (1996)). After incubation at 4°C for 15 minutes, 0.25 ml of the TK1 cell suspension (5×10^5 TK1 cells) was added to each well and incubation on a rocking platform was continued for an additional 30 minutes at 4°C. Plates were washed by gently
30 inverting in a large beaker of phosphate buffered saline (PBS) followed by inversion in a beaker of PBS with 1.5% glutaraldehyde for fixation for a minimum of 1 hour. Wells were then examined microscopically (10X objective) for rosetting of TK1 cells mediated by the pools of cdNA

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clones. Pools yielding one or more TK1 rosettes were further subfractionated three times until individual colonies could be assayed and the clones conferring adhesion of the TK1 cells were identified.

5 Functional Adhesion Assays

Assays with purified clones were similar to those performed in expression cloning with the following exception: as several wells were to be transfected for antibody inhibition studies, a master liposome mix with multiples of the wells to be transfected was first made for each plasmid. On the day of the assay monoclonal antibodies were incubated with cells at 20 μ g/ml or supernatants (undiluted) at 4°C for 15 minutes prior to the start of the assay.

For adhesion assays with hyaluronan, human umbilical cord hyaluronan (Calbiochem, San Diego, CA) was diluted to 5 mg/ml in PBS. *Streptomyces* hyaluronidase (Calbiochem, San Diego, CA) was diluted to 20 TRU/ml in HBSS. TK1 cells were resuspended in HBSS containing 2 mM CaCl₂, 2 mM MgCl₂, 2% serum and 20 mM HEPES at 10⁶ cells/ml. Wells of 24-well plates were coated with 200 μ l of hyaluronan and stored at 4°C overnight. Wells were rinsed with 0.5 ml PBS three times, and were treated with 0.25 ml *Streptomyces* hyaluronidase at final concentrations of 0, 5, 10 and 20 TRU/ml for 1 hour at 37°C. Wells were rinsed three times with 0.5 ml PBS, blocked with 0.5 ml serum for 1 hour on ice and then rinsed three times with 0.5 ml PBS. TK1 cells (0.5 ml) were added to each well and plates were incubated with shaking at 4°C for 20 minutes.

For assessment of hyaluronate mediated binding to CHO/P cells, the transfectants were rinsed with 0.5 ml PBS three times. Individual wells were treated with 250 μ l *Streptomyces* hyaluronidase at 0, 5, 10 and 20 TRU/ml (final

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concentrations) for 1 hour at 37°C. Transfectants were rinsed three times with 0.5 ml PBS. TK1 cells (0.5 ml in the same buffer as described above) were added to each well and plates were incubated with shaking at 4°C for 30 minutes. Wells were rinsed with 0.5 ml PBS three times and viewed under the light microscope. Assays were fixed as described above and analyzed by examination of multiple fields and counting both lymphocytes and CHO cells at 10X magnification.

10 Measurement of Hyaluronic Acid Biosynthesis in CHO Cell Transfectants

0.5 x 10⁶ CHO cells seeded in 100mm plates were transfected with Lipofectamine reagent according to manufacturer's instructions. Transfections utilized 20µg of HAS cDNA in pCDNA3 (Invitrogen, San Diego, CA) and 160 µl of lipofectamine reagent. Clone 30C was digested with EcoRI and NotI and the insert released thereby was cloned into the EcoRI and NotI sites of pCDNA3. Transformants of *E. coli* XL-1 Blue (Stratagene) or DH10B (Gibco) containing the resulting construct were obtained. Approximately 72 hours after transfection, 440 µg/ml of G418 was added in fresh media. After the transfected and control (non transfected) cells had reached subconfluency, the media was replaced with fresh complete media containing 5 mCi/ml D-[6-³H] glucosamine hydrochloride (New England Nuclear, Boston, MA, specific activity 33.3 ci/ml, concentration 1mCi/ml), a precursor of sulfated glucosaminoglycans such as hyaluronan. The amounts of synthesized hyaluronan in transfected and control CHO cells were determined after 48 hours of incubation at 37°C as follows. Media was collected and the cell layers were combined with the corresponding media. Aliquots from each sample were incubated overnight at 37°C in the presence or

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absence of *Streptomyces* hyaluronidase. Then the samples were applied on sephadex G-50 superfine columns (100 X 100mm) which were equilibrated with 0.05 M sodium acetate, pH 6.0 containing 0.2M NaCl. Newly synthesized [³H]

- 5 hyaluronan was determined as the *Streptomyces* sensitive radioactivity.

DNA Sequencing

- Plasmids were sequenced on both strands using oligonucleotide primers and the sequenase™ 7-deaza-dGTP DNA
10 sequencing kit with sequenase version 2.0 T7 DNA polymerase (United States Biochemical, Cleveland, OH) and ³⁵SdCTP (Amersham Life Science, Arlington Heights, IL and New England Nuclear, Boston, MA) using manufacturer's instructions.

15 Northern and Southern Blot Analysis

- Northern blots used were human multiple tissue northern I and II (Clontech, Palo Alto, CA). Hybridization was performed with ExpressHyb (Clontech) solution, using manufacturer's instructions except that a
20 final wash at high stringency (0.1X SSC, 0.1% SDS, 65°C) for 30 min was added. A commercially prepared southern blot (Human GENO-BLOT) (Clontech, Palo Alto, CA) was hybridized as described for the Northern blot with the exception that an initial wash at 50°C was exposed and then
25 the blot was subsequently washed at 65°C and exposed again. cDNA's were labelled with α³²P-dCTP by priming with random hexamers. After washing, filters were exposed to Kodak XAR film with an intensifying screen.

RESULTS AND DISCUSSION

An expression cloning system was developed to isolate cDNA clones that encode proteins that confer adhesion of the murine T cell lymphoma TK1 (Butcher, E.C. et al., *Eur. J. Immunol.* 10:556-561 (1980)). A human mesenteric lymph node expression library was constructed that, upon transfection into CHO/P cells, yielded a cDNA clone, called 30C, that mediated rosetting of TK1 cells to some of the CHO/P transfectants. Transformants of *E. coli* XL-1 Blue (Stratagene) containing Clone 30C were obtained. In order to understand the nature of the observed interaction, the adhesion assay after pre-incubation of the TK1 line with several antibodies to adhesion receptors known to be expressed on TK1 cells was repeated. Binding could be completely inhibited by pre-incubation of TK1 cells with an antibody to CD44 (Table 1), while other antibodies (anti- α 4 and anti- β 7 integrins (Andrew, D.P. et al., *J. Immunol.* 153:3847-3861 (1994); Miyake, K. *J. Exp. Med.* 173:599-607 (1991)) had no effect.

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Cells/Matrix	TK1 Cell	TK1 Binding after hyaluronidase	TK1 Binding after anti-CD44 Mab TJB1.7	TK1 Binding after anti-a4 Mab PS/2
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	HAS	+++	-	-	+++
	Transfectants				
	Mock	-	-	-	-
5	Transfectants				
	Hyaluronate	+++	-	-	+++

Table 1. Adhesion of TK1 cells to clone 30C transfectants. TK1 cells bind to CHO/P cells transiently transfected with clone 30C. Binding is blocked by pretreatment of the
 10 transfectants with hyaluronidase or pretreatment of TK1 cells with anti-CD44 Mab TJB1.7. Similar results are seen with binding to immobilized hyaluronate, while TK1 cells do not bind mock transfectants. A score of "-" indicates that
 15 no TK1 cells (above controls) were observed in those wells while "+++" indicates TK1 rosetting on transfectants (> 5 TK1 cells/CHO/P transfectant) or a monolayer of cells binding to immobilized hyaluronate. Assays were all repeated three times with similar results.

As CD44 is known to be a hyaluronan receptor (Aruffo, A.,
 20 et al., *Cell* 61:1303-1313 (1990); Culty, M. et al., *J. Cell. Biol.*, 111:2765-2774 (1990); Miyake, K. et al., *J. Exp. Med.* 172:69-75 (1990)), it was investigated whether the isolated cDNA encoded a novel CD44 ligand or, alternatively, was involved in *de novo* synthesis of
 25 hyaluronan. Hyaluronidase pretreatment completely abrogated TK1 binding to the transfectants as well as to hyaluronan controls (Table 1), indicating that the cloned cDNA mediated synthesis of HA. Finally, CHO cells were stably transfected with the 30C cDNA and assessed for their
 30 ability to mediate hyaluronan biosynthesis (Figure 1A,B).

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Whereas, untransfected cells produced very little high molecular weight *Streptomyces* hyaluronidase-sensitive material (Figure 1B), cell cultures transfected with 30C cDNA produced a substantial amount of hyaluronan

5 (Figure 1A).

The cDNA encoding clone 30C is 2116 nucleotides in length (Figure 2) with a short 5' untranslated region of 35 bp and a longer 3' untranslated region of 347 bp. From the first ATG, a predicted open reading frame of 1734 bp
10 yielding a protein of 578 amino acid residues is present. Genbank searches of the nucleotide and protein sequences revealed significant homology with the hasA gene of *Streptococcus pyogenes* (DeAngelis, J.P.a.P.H.W., *J. Biol. Chem.* 268:19181-19184 (1993)), which was reported to be a
15 hyaluronan synthase (Figure 3A-3B) and a sequence from *Xenopus laevis* called DG42 (Figure 3A-3B) which has also been speculated to be a glycosaminoglycan synthetase (Rosa, F. et al., *Develop. Biol.* 129:114-123 (1988)). Amino acid sequence identities between the predicted protein and these
20 sequences were 22% and 54%, respectively. Significant similarity was also observed with other membrane associated proteins with N-acetylglycosylamino transferase activity including NodC from *Rhizobium* and three chitin synthases from *Saccharomyces* (Chs) (DeAngelis, P.L. et al., *Biochem.*
25 *and Biophys. Res. Comm.* 199:1-10 (1994)). The similarities observed, coupled with the functional adhesion indicate that clone 30C encodes a human homolog of hyaluronan synthase (HS). Using nomenclature based on the streptococcus gene locus, this human gene encoding
30 hyaluronan synthase is designated HAS.

The predicted molecular mass of the HAS protein is 64,793 daltons. Hydrophilicity (Kyte-Doolittle) analysis predicts a membrane protein with several hydrophobic regions that would be predicted to span the cell membrane
35 at least four times (Figure 3A-3C). This prediction is in agreement with labeling studies which suggested that

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hyaluronan synthase is associated with the plasma membrane (Prehm, P., *Biochem. J.* 220:597-600 (1984); Phillipson, L.H. and Schwartz, N.B. *J. Biol. Chem.* 259:5017-5023 (1984); Klewes, L. et al., *Biochem J.* 290:791-795 (1993);
5 O'Regan, M. et al., *Int. J. Biol. Macromol.* 16:283-286 (1994)). Conservation of secondary structure between hasA, DG42 and HAS, is indicated by similar hydrophilicity plots. The approximate locations of these regions, with respect to
10 representative hydrophilicity plots are shown in Figure 3B.

The estimated number of transmembrane segments would suggest a structure with a small N-terminal extracellular domain followed by a long intracellular loop and then three
15 more transmembrane regions to yield one more small extracellular loop, a small intracellular loop followed by a C-terminal extracellular extension (Figure 3C). Such a model, with the predominant portion of the protein located intracellularly would be consistent with studies indicating that hyaluronan biosynthesis occurs at the inner surface of
20 the plasma membrane (Prehm, P. *Biochem. J.* 220:597-600 (1984); Phillipson, L.H., and Schwartz, N.B. *J. Biol. Chem.* 259:5017-5023 (1984)). This predicted large intercellular loop, is more highly conserved than the overall protein at 70% (vs 54%) when compared with DG42, which would imply
25 conservation of a functional domain. Within the amino terminal portion of this domain lies a motif, designated B(X₇)B (Figures 2 and 3C), where B is a basic amino acid (e.g., R, K) and X is any non-acidic residue. This motif has been found in both RHAMM, link protein and CD44, and
30 mutagenesis studies has shown that this sequence is required for binding hyaluronan (Yang, B., et al., *EMBO* 13:286-296 (1994)). The presence of this putative hyaluronan binding motif (HBM) in HAS raises the possibility of a requirement of binding hyaluronan during
35 its synthesis and prior to transport out of the cell.

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Northern blots probed with the entire human cDNA, revealed a major transcript of 2.4 kb that was most highly expressed in ovary and also expressed at significant levels in spleen, thymus, prostate, testes and large intestine (Figure 4A). In addition, a less abundant transcript of approximately 7 kb was also observed in these tissues and in addition to a faint 9 kb species only expressed in ovary. Extremely weak expression was observed in small intestine while peripheral blood leukocytes (PBL) were negative under the conditions used. Moderate expression was also observed in heart. The larger transcript observed might be a related gene in these tissues although a southern blot probed first with both full length and then a 3' region of HAS cDNA and washed at several temperatures shows a simple banding pattern suggestive of a single copy gene (Figure 4B). It is therefore likely that these larger species represent unprocessed nuclear precursors, as opposed to related genes. The expression pattern observed is consistent with high levels of hyaluronan that are observed in lymphoid tissues, preovulatory follicles and in perivascular connective tissue and vessel walls of both atrium and ventricle (Edelstrom, G.A.B. et al., *Histochem. Cytochem.*, 39:1131-1135 (1991); Laurent, C. et al., *Cell Tissue Res.*, 263: 201-205 (1991)) and would indicate that synthesis of hyaluronan is at least partially regulated by transcriptional mechanisms. Interestingly, however, expression of HAS RNA was barely detectable in skeletal muscle under the conditions used, although histochemical analysis has shown ubiquitous distribution of hyaluronan in connective tissue and the septum dividing muscle fibers (Edelstrom, G.A.B. et al., *Histochem. Cytochem* 39:1131-1135 (1991); Laurent, C. et al., *Cell Tissue Res.* 263: 201-205 (1991)). This may indicate that turnover rates of hyaluronan may display great variation in different tissues.

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Induction of synthase activity by growth factors has been shown to require protein synthesis and is mediated by a signaling pathway involving tyrosine phosphorylation and/or activation of protein kinase C (Heldin, P. et al., *Biochem. J.* 258, 919-922 (1992); Suzuki, M. et al., *Biochem. J.* 307:817-821 (1995)) as both PMA and inhibitors of phosphotyrosine phosphatases can induce hyaluronan synthesis. Serum alone can also induce synthase activity and this induction was blocked by protein kinase C inhibitors and cycloheximide. cAMP has also been implicated in activation and phosphorylation of the synthase itself may play a key role in regulation of its activity (Klewes, L. and Prehm, P., *J. of Cell. Physiol.* 160:539-544 (1994)). Examination of hydrophilic regions of HAS reveals several conserved motifs which are potential substrates for protein kinase C and cAMP dependent kinases (Figures 2, 3C.) and are likely targets for future mutagenesis studies (Pearson, R.B. *Studies of protein kinase/phosphatase specificity using synthetic peptides*. Protein phosphorylation: A practical approach (Hardie, D.G., Ed.), Oxford University Press, Oxford (1993)). As observed, increased expression of the HAS gene in tissues that are known to produce large quantities of hyaluronan, it is likely that the regulation of hyaluronan synthesis is mediated by regulation of HAS gene transcription, in addition to complex regulatory circuits which involve both alterations in phosphorylation of the synthase or proteins associated with HAS.

Previously, a 52 kDa protein was isolated from a mouse/hamster hybridoma (B6 cells) that was initially reported to be a mammalian hyaluronan synthase (Klewes, L. et al., *Biochem J.* 290:791-795 (1993)). This protein was incapable of binding UDP-Glucuronic acid (UDP-[14C] GlcA) and UDP-N-acetyl glucosamine (UDP-[3H] GlcNAc) unless complexed to a 60 kDa protein, which may be the hyaluronan receptor (RHAMM) recently implicated in fibroblast migration and tumor metastasis (Turley, E.A. et al., *J.*

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Cell Biol., 112:1041-1047 (1991)). This protein cross-reacted with antibodies against a putative synthase from *Streptococcus equisimilis*. The gene encoding this protein was cloned from a streptococcal library and shown to be
5 related to proteins involved in oligopeptide processing and transport and showed no homology to the *hasA* gene sequence (O'Regan, M. et al., *Int. J. Biol. Macromol.* 16:283-286 (1994); Lansing, M. et al., *Biochem. J.* 289:179-184 (1993)). It is likely that the 52 kd protein isolated from
10 the B6 line is a homolog to the streptococcal transport protein and not the synthase itself. The human hyaluronan synthase cDNA is therefore the first example of a mammalian gene responsible for synthesis of hyaluronan.

Studies in streptococci show that the machinery
15 responsible for synthesis of hyaluronan is encoded in the *has* operon which consists of three genes *hasA*, B and C (Dougherty, B.P., and van de Rijn, I. *J. Biol. Chem.* 269:169-175 (1994); Dougherty, B.P., and van de Rijn, I. *J. Biol. Chem.* 268:7118-7124 (1993); Crater, D.L., and van de
20 Rijn, I. *J. Biol. Chem.* 270:18452-18458 (1995)). It has been demonstrated that HAS is homologous to *hasA* which encodes hyaluronan synthase. The *hasB* and C loci encode UDP:Glc dehydrogenase and UDP-GLc pyrophosphorylase respectively (Dougherty, B.P., and van de Rijn, I. *J. Biol.*
25 *Chem.* 269:169-175 (1994); Dougherty, B.P., and van de Rijn, I. *J. Biol. Chem.* 268:7118-7124 (1993); Crater, D.L., and van de Rijn, I. *J. Biol. Chem.* 270:18452-18458 (1995)). Also demonstrated herein is that transfection of the HAS cDNA into CHO cells is sufficient to mediate *de novo*
30 synthesis of hyaluronan, which indicates that all of the other factors necessary for hyaluronan biosynthesis such as those encoded by *hasB* and C are possibly expressed in CHO cells. Recent data suggests that hyaluronan can also be synthesized upon transfection of the synthase into COS
35 cells and a murine preB lymphoma which suggests that these backgrounds have endogenous UDP-GLc dehydrogenase and

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UDP-GLc phosphorylase and expression of HAS is then the most significant factor in regulating hyaluronan synthesis in mammalian cells. The identification of this cDNA will therefore assist further characterization of the molecular events resulting in synthesis of hyaluronan and its relationship to cellular migration in wound healing, tumor metastasis and leukocyte migration.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

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(ii) TITLE OF INVENTION: MAMMALIAN HYALURONAN SYNTHASES, NUCLEIC ACIDS, USES THEREOF

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2116 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 36..1769

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGGAGAGAAG AGAGAGCCCG GCCAGACCCA CTGCG ATG AGA CAG CAG GAC GCG	53
Met Arg Gln Gln Asp Ala	
1 5	
CCC AAG CCC ACT CCT GCA GCC CGC CGC TGC TCC GGC CTG GCC CGG AGG	101
Pro Lys Pro Thr Pro Ala Ala Arg Arg Cys Ser Gly Leu Ala Arg Arg	
10 15 20	
GTG CTG ACC ATC GCC TTC GCC CTG CTC ATC CTG GGC CTC ATG ACC TGG	149
Val Leu Thr Ile Ala Phe Ala Leu Leu Ile Leu Gly Leu Met Thr Trp	
25 30 35	
GCC TAC GCC GCC GGG GTG CCG CTG GCC TCC GAT CGC TAC GGC CTC CTG	197
Ala Tyr Ala Ala Gly Val Pro Leu Ala Ser Asp Arg Tyr Gly Leu Leu	
40 45 50	
GCC TTC GGC CTC TAC GGG GCC TTC CTT TCA GCG CAC CTG GTG GCG CAG	245
Ala Phe Gly Leu Tyr Gly Ala Phe Leu Ser Ala His Leu Val Ala Gln	
55 60 65 70	
AGC CTC TTC GCG TAC CTG GAG CAC CGG CGG GTG GCG GCG GCG GCG CGG	293
Ser Leu Phe Ala Tyr Leu Glu His Arg Arg Val Ala Ala Ala Ala Arg	
75 80 85	
GGG CCG CTG GAT GCA GCC ACC GCG CGC AGT GTG GCG CTG ACC ATC TCC	341
Gly Pro Leu Asp Ala Ala Thr Ala Arg Ser Val Ala Leu Thr Ile Ser	
90 95 100	
GCC TAC CAG GAG GAC CCC GCG TAC CTG CGC CAG TGC CTG GCG TCC GCC	389
Ala Tyr Gln Glu Asp Pro Ala Tyr Leu Arg Gln Cys Leu Ala Ser Ala	
105 110 115	
CGC GCC CTG CTG TAC CCG CGC GCG CGG CTG CGC GTC CTC ATG GTG GTG	437
Arg Ala Leu Leu Tyr Pro Arg Ala Arg Leu Arg Val Leu Met Val Val	
120 125 130	
GAT GGC AAC CGC GCC GAG GAC CTC TAC ATG GTC GAC ATG TTC CGC GAG	485
Asp Gly Asn Arg Ala Glu Asp Leu Tyr Met Val Asp Met Phe Arg Glu	
135 140 145 150	
GTC TTC GCT GAC GAG GAC CCC GCC ACG TAC GTG TGG GAC GGC AAC TAC	533
Val Phe Ala Asp Glu Asp Pro Ala Thr Tyr Val Trp Asp Gly Asn Tyr	
155 160 165	
CAC CAG CCC TGG GAA CCC GCG GCG GCG GGC GCG GTG GGC GCC GGA GCC	581
His Gln Pro Trp Glu Pro Ala Ala Ala Gly Ala Val Gly Ala Gly Ala	
170 175 180	
TAT CGG GAG GTG GAG GCG GAG GAT CCT GGG CGG CTG GCA GTG GAG GCG	629
Tyr Arg Glu Val Glu Ala Glu Asp Pro Gly Arg Leu Ala Val Glu Ala	
185 190 195	

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CTG	GTG	AGG	ACT	CGC	AGG	TGC	GTG	TGC	GTG	GCG	CAG	CGC	TGG	GGC	GGC	677
Leu	Val	Arg	Thr	Arg	Arg	Cys	Val	Cys	Val	Ala	Gln	Arg	Trp	Gly	Gly	
200						205					210					
AAG	CGC	GAG	GTC	ATG	TAC	ACA	GCC	TTC	AAG	GCG	CTC	GGA	GAT	TCG	GTG	725
Lys	Arg	Glu	Val	Met	Tyr	Thr	Ala	Phe	Lys	Ala	Leu	Gly	Asp	Ser	Val	
215					220					225					230	
GAC	TAC	GTG	CAG	GTC	TGT	GAC	TCG	GAC	ACA	AGG	TTG	GAC	CCC	ATG	GCA	773
Asp	Tyr	Val	Gln	Val	Cys	Asp	Ser	Asp	Thr	Arg	Leu	Asp	Pro	Met	Ala	
				235					240					245		
CTG	CTG	GAG	CTC	GTG	CGG	GTA	CTG	GAC	GAG	GAC	CCC	CGG	GTA	GGG	GCT	821
Leu	Leu	Glu	Leu	Val	Arg	Val	Leu	Asp	Glu	Asp	Pro	Arg	Val	Gly	Ala	
			250					255					260			
GTT	GGT	GGG	GAC	GTG	CGG	ATC	CTT	AAC	CCT	CTG	GAC	TCC	TGG	GTC	AGC	869
Val	Gly	Gly	Asp	Val	Arg	Ile	Leu	Asn	Pro	Leu	Asp	Ser	Trp	Val	Ser	
		265					270					275				
TTC	CTA	AGC	AGC	CTG	CGA	TAC	TGG	GTA	GCC	TTC	AAT	GTG	GAG	CGG	GCT	917
Phe	Leu	Ser	Ser	Leu	Arg	Tyr	Trp	Val	Ala	Phe	Asn	Val	Glu	Arg	Ala	
	280					285					290					
TGT	CAG	AGC	TAC	TTC	CAC	TGT	GTA	TCC	TGC	ATC	AGC	GGT	CCT	CTA	GGC	965
Cys	Gln	Ser	Tyr	Phe	His	Cys	Val	Ser	Cys	Ile	Ser	Gly	Pro	Leu	Gly	
295					300					305					310	
CTA	TAT	AGG	AAT	AAC	CTC	TTG	CAG	CAG	TTT	CTT	GAG	GCC	TGG	TAC	AAC	1013
Leu	Tyr	Arg	Asn	Leu	Leu	Gln	Gln	Phe	Leu	Glu	Ala	Trp	Tyr	Asn		
			315					320						325		
CAG	AAG	TTC	CTG	GGT	ACC	CAC	TGT	ACT	TTT	GGG	GAT	GAC	CGG	CAC	CTC	1061
Gln	Lys	Phe	Leu	Gly	Thr	His	Cys	Thr	Phe	Gly	Asp	Asp	Arg	His	Leu	
			330					335					340			
ACC	AAC	CGC	ATG	CTC	AGC	ATG	GGT	TAT	GCT	ACC	AAG	TAC	ACC	TCC	AGG	1109
Thr	Asn	Arg	Met	Leu	Ser	Met	Gly	Tyr	Ala	Thr	Lys	Tyr	Thr	Ser	Arg	
		345					350					355				
TCC	CGC	TGC	TAC	TCA	GAG	ACG	CCC	TCG	TCC	TTC	CTG	CGG	TGG	CTG	AGC	1157
Ser	Arg	Cys	Tyr	Ser	Glu	Thr	Pro	Ser	Ser	Phe	Leu	Arg	Trp	Leu	Ser	
	360					365					370					
CAG	CAG	ACA	CGC	TGG	TCC	AAG	TCG	TAC	TTC	CGT	GAG	TGG	CTG	TAC	AAC	1205
Gln	Gln	Thr	Arg	Trp	Ser	Lys	Ser	Tyr	Phe	Arg	Glu	Trp	Leu	Tyr	Asn	
375					380					385					390	
GCG	CTC	TGG	TGG	CAC	CGG	CAC	CAT	GCG	TGG	ATG	ACC	TAC	GAG	GCG	GTG	1253
Ala	Leu	Trp	Trp	His	Arg	His	His	Ala	Trp	Met	Thr	Tyr	Glu	Ala	Val	
				395					400					405		
GTC	TCC	GGC	CTG	TTC	CCC	TTC	TTC	GTG	GCG	GCC	ACT	GTG	CTG	CGT	CTG	1301
Val	Ser	Gly	Leu	Phe	Pro	Phe	Phe	Val	Ala	Ala	Thr	Val	Leu	Arg	Leu	
			410					415					420			
TTC	TAC	GCG	GGC	CGC	CCT	TGG	GCG	CTG	CTG	TGG	GTG	CTG	CTG	TGC	GTG	1349
Phe	Tyr	Ala	Gly	Arg	Pro	Trp	Ala	Leu	Leu	Trp	Val	Leu	Leu	Cys	Val	
		425					430					435				

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CAG GGC GTG GCA CTG GCC AAG GCG GCC TTC GCG GCC TGG CTG CGG GGC Gln Gly Val Ala Leu Ala Lys Ala Ala Phe Ala Ala Trp Leu Arg Gly 440 445 450	1397
TGC CTG CGC ATG GTG CTT CTG TCG CTC TAC GCG CCC CTC TAC ATG TGT Cys Leu Arg Met Val Leu Ser Leu Tyr Ala Pro Leu Tyr Met Cys 455 460 465 470	1445
GGC CTC CTG CCT GCC AAG TTC CTG GCG CTA GTC ACC ATG AAC CAG AGT Gly Leu Leu Pro Ala Lys Phe Leu Ala Leu Val Thr Met Asn Gln Ser 475 480 485	1493
GGC TGG GGC ACC TCG GGC CGG CGG AAG CTG GCC GCT AAC TAC GTC CCT Gly Trp Gly Thr Ser Gly Arg Arg Lys Leu Ala Ala Asn Tyr Val Pro 490 495 500	1541
CTG CTG CCC CTG GCG CTC TGG GCG CTG CTG CTG CTT GGG GGC CTG GTC Leu Leu Pro Leu Ala Leu Trp Ala Leu Leu Leu Leu Gly Gly Leu Val 505 510 515	1589
CGC AGC GTA GCA CAC GAG GCC AGG GCC GAC TGG AGC GGC CCT TCC CGC Arg Ser Val Ala His Glu Ala Arg Ala Asp Trp Ser Gly Pro Ser Arg 520 525 530	1637
GCA GCC GAG GCC TAC CAC TTG GCC GCG GGG GCC GGC GCC TAC GTG GGC Ala Ala Glu Ala Tyr His Leu Ala Ala Gly Ala Gly Ala Tyr Val Gly 535 540 545 550	1685
TAC TGG GTG GCC ATG TTG ACG CTG TAC TGG GTG GGC GTG CGG AGG CTT Tyr Trp Val Ala Met Leu Thr Leu Tyr Trp Val Gly Val Arg Arg Leu 555 560 565	1733
TGC CGG CGG CGG ACC GGG GGC TAC CGC GTC CAG GTG TGAGTCCAGC Cys Arg Arg Arg Thr Gly Gly Tyr Arg Val Gln Val 570 575	1779
CACGCGGATG CCGCCTCAAG GGTCTTCAGG GGAGGCCAGA GGAGAGCTGC TGGGCCCCGA	1839
GCCACGAACT TGCTGGGTGG TTCTCTGGGC CTCAGTTTCC CTCCTCTGCC AAACGAGGGG	1899
GTCAGCCCAA GATTCTTCAG TCTGGAATAT ATTGGGACTG GGAATTCTGG GTCTCCAGGG	1959
AGGGTATTTA TTGGTCAGGA TGTGGGATTT GAGGAGTGGG GGGGAAAGGG TCCTGCTTTC	2019
TCCTCGTTCT TATTTAATCT CCATTTCTAC TGTGTGATCA GGATGTAATA AAGAATTTTA	2079
TTTATTTTCA AAAAAAAAAA AAAAAAAAAA AAAAAAA	2116

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 578 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Arg	Gln	Gln	Asp	Ala	Pro	Lys	Pro	Thr	Pro	Ala	Ala	Arg	Arg	Cys
1				5					10					15	

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Ser Gly Leu Ala Arg Arg Val Leu Thr Ile Ala Phe Ala Leu Leu Ile
 20 25 30
 Leu Gly Leu Met Thr Trp Ala Tyr Ala Ala Gly Val Pro Leu Ala Ser
 35 40 45
 Asp Arg Tyr Gly Leu Leu Ala Phe Gly Leu Tyr Gly Ala Phe Leu Ser
 50 55 60
 Ala His Leu Val Ala Gln Ser Leu Phe Ala Tyr Leu Glu His Arg Arg
 65 70 75 80
 Val Ala Ala Ala Ala Arg Gly Pro Leu Asp Ala Ala Thr Ala Arg Ser
 85 90 95
 Val Ala Leu Thr Ile Ser Ala Tyr Gln Glu Asp Pro Ala Tyr Leu Arg
 100 105 110
 Gln Cys Leu Ala Ser Ala Arg Ala Leu Leu Tyr Pro Arg Ala Arg Leu
 115 120 125
 Arg Val Leu Met Val Val Asp Gly Asn Arg Ala Glu Asp Leu Tyr Met
 130 135 140
 Val Asp Met Phe Arg Glu Val Phe Ala Asp Glu Asp Pro Ala Thr Tyr
 145 150 155 160
 Val Trp Asp Gly Asn Tyr His Gln Pro Trp Glu Pro Ala Ala Ala Gly
 165 170 175
 Ala Val Gly Ala Gly Ala Tyr Arg Glu Val Glu Ala Glu Asp Pro Gly
 180 185 190
 Arg Leu Ala Val Glu Ala Leu Val Arg Thr Arg Arg Cys Val Cys Val
 195 200 205
 Ala Gln Arg Trp Gly Gly Lys Arg Glu Val Met Tyr Thr Ala Phe Lys
 210 215 220
 Ala Leu Gly Asp Ser Val Asp Tyr Val Gln Val Cys Asp Ser Asp Thr
 225 230 235 240
 Arg Leu Asp Pro Met Ala Leu Leu Glu Leu Val Arg Val Leu Asp Glu
 245 250 255
 Asp Pro Arg Val Gly Ala Val Gly Gly Asp Val Arg Ile Leu Asn Pro
 260 265 270
 Leu Asp Ser Trp Val Ser Phe Leu Ser Ser Leu Arg Tyr Trp Val Ala
 275 280 285
 Phe Asn Val Glu Arg Ala Cys Gln Ser Tyr Phe His Cys Val Ser Cys
 290 295 300
 Ile Ser Gly Pro Leu Gly Leu Tyr Arg Asn Asn Leu Leu Gln Gln Phe
 305 310 315 320
 Leu Glu Ala Trp Tyr Asn Gln Lys Phe Leu Gly Thr His Cys Thr Phe
 325 330 335
 Gly Asp Asp Arg His Leu Thr Asn Arg Met Leu Ser Met Gly Tyr Ala
 340 345 350

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Thr Lys Tyr Thr Ser Arg Ser Arg Cys Tyr Ser Glu Thr Pro Ser Ser
 355 360 365
 Phe Leu Arg Trp Leu Ser Gln Gln Thr Arg Trp Ser Lys Ser Tyr Phe
 370 375 380
 Arg Glu Trp Leu Tyr Asn Ala Leu Trp Trp His Arg His His Ala Trp
 385 390 395 400
 Met Thr Tyr Glu Ala Val Val Ser Gly Leu Phe Pro Phe Phe Val Ala
 405 410 415
 Ala Thr Val Leu Arg Leu Phe Tyr Ala Gly Arg Pro Trp Ala Leu Leu
 420 425 430
 Trp Val Leu Leu Cys Val Gln Gly Val Ala Leu Ala Lys Ala Ala Phe
 435 440 445
 Ala Ala Trp Leu Arg Gly Cys Leu Arg Met Val Leu Leu Ser Leu Tyr
 450 455 460
 Ala Pro Leu Tyr Met Cys Gly Leu Leu Pro Ala Lys Phe Leu Ala Leu
 465 470 475 480
 Val Thr Met Asn Gln Ser Gly Trp Gly Thr Ser Gly Arg Arg Lys Leu
 485 490 495
 Ala Ala Asn Tyr Val Pro Leu Leu Pro Leu Ala Leu Trp Ala Leu Leu
 500 505 510
 Leu Leu Gly Gly Leu Val Arg Ser Val Ala His Glu Ala Arg Ala Asp
 515 520 525
 Trp Ser Gly Pro Ser Arg Ala Ala Glu Ala Tyr His Leu Ala Ala Gly
 530 535 540
 Ala Gly Ala Tyr Val Gly Tyr Trp Val Ala Met Leu Thr Leu Tyr Trp
 545 550 555 560
 Val Gly Val Arg Arg Leu Cys Arg Arg Arg Thr Gly Gly Tyr Arg Val
 565 570 575
 Gln Val

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 587 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Lys Glu Lys Ala Ala Glu Thr Met Glu Ile Pro Glu Gly Ile Pro
 1 5 10 15

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Lys Asp Leu Glu Pro Lys His Pro Thr Leu Trp Arg Ile Ile Tyr Tyr
 20 25 30
 Ser Phe Gly Val Val Leu Leu Ala Thr Ile Thr Ala Ala Tyr Val Ala
 35 40 45
 Glu Phe Gln Val Leu Lys His Glu Ala Ile Leu Phe Ser Leu Gly Leu
 50 55 60
 Tyr Gly Leu Ala Met Leu Leu His Leu Met Met Gln Ser Leu Phe Ala
 65 70 75 80
 Phe Leu Glu Ile Arg Arg Val Asn Lys Ser Glu Leu Pro Cys Ser Phe
 85 90 95
 Lys Lys Thr Val Ala Leu Thr Ile Ala Gly Tyr Gln Glu Asn Pro Glu
 100 105 110
 Tyr Leu Ile Lys Cys Leu Glu Ser Cys Lys Tyr Val Lys Tyr Pro Lys
 115 120 125
 Asp Lys Leu Lys Ile Ile Leu Val Ile Asp Gly Asn Thr Glu Asp Asp
 130 135 140
 Ala Tyr Met Met Glu Met Phe Lys Asp Val Phe His Gly Glu Asp Val
 145 150 155 160
 Gly Thr Tyr Val Trp Lys Gly Asn Tyr His Thr Val Lys Lys Pro Glu
 165 170 175
 Glu Thr Asn Lys Gly Ser Cys Pro Glu Val Ser Lys Pro Leu Asn Glu
 180 185 190
 Asp Glu Gly Ile Asn Met Val Glu Glu Leu Val Arg Asn Lys Arg Cys
 195 200 205
 Val Cys Ile Met Gln Gln Trp Gly Gly Lys Arg Glu Val Met Tyr Thr
 210 215 220
 Ala Phe Gln Ala Ile Gly Thr Ser Val Asp Tyr Val Gln Val Cys Asp
 225 230 235 240
 Ser Asp Thr Lys Leu Asp Glu Leu Ala Thr Val Glu Met Val Lys Val
 245 250 255
 Leu Glu Ser Asn Asp Met Tyr Gly Ala Val Gly Gly Asp Val Arg Ile
 260 265 270
 Leu Asn Pro Tyr Asp Ser Phe Ile Ser Phe Met Ser Ser Leu Arg Tyr
 275 280 285
 Trp Met Ala Phe Asn Val Glu Arg Ala Cys Gln Ser Tyr Phe Asp Cys
 290 295 300
 Val Ser Cys Ile Ser Gly Pro Leu Gly Met Tyr Arg Asn Asn Ile Leu
 305 310 315 320
 Gln Val Phe Leu Glu Ala Trp Tyr Arg Gln Lys Phe Leu Gly Thr Tyr
 325 330 335

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Cys Thr Leu Gly Asp Asp Arg His Leu Thr Asn Arg Val Leu Ser Met
 340 345 350
 Gly Tyr Arg Thr Lys Tyr Thr His Lys Ser Arg Ala Phe Ser Glu Thr
 355 360 365
 Pro Ser Leu Tyr Leu Arg Trp Leu Asn Gln Gln Thr Arg Trp Thr Lys
 370 375 380
 Ser Tyr Phe Arg Glu Trp Leu Tyr Asn Ala Gln Trp Trp His Lys His
 385 390 395 400

 His Ile Trp Met Thr Tyr Glu Ser Val Val Ser Phe Ile Phe Pro Phe
 405 410 415
 Phe Ile Thr Ala Thr Val Ile Arg Leu Ile Tyr Ala Gly Thr Ile Trp
 420 425 430
 Asn Val Val Trp Leu Leu Leu Cys Ile Gln Ile Met Ser Leu Phe Lys
 435 440 445
 Ser Ile Tyr Ala Cys Trp Leu Arg Gly Asn Phe Ile Met Leu Leu Met
 450 455 460
 Ser Leu Tyr Ser Met Leu Tyr Met Thr Gly Leu Leu Pro Ser Lys Tyr
 465 470 475 480
 Phe Ala Leu Leu Thr Leu Asn Lys Thr Gly Trp Gly Thr Gly Arg Lys
 485 490 495
 Lys Ile Val Gly Asn Tyr Met Pro Ile Leu Pro Leu Ser Ile Trp Ala
 500 505 510
 Ala Val Leu Cys Gly Gly Val Gly Tyr Ser Ile Tyr Met Asp Cys Gln
 515 520 525
 Asn Asp Trp Ser Thr Pro Glu Lys Gln Lys Glu Met Tyr His Leu Leu
 530 535 540
 Tyr Gly Cys Val Gly Tyr Val Met Tyr Trp Val Ile Met Ala Val Met
 545 550 555 560
 Tyr Trp Val Trp Val Lys Arg Cys Cys Arg Lys Arg Ser Gln Thr Val
 565 570 575
 Thr Leu Val His Asp Ile Pro Asp Met Cys Val
 580 585

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 395 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Tyr	Leu	Phe	Gly	Thr	Ser	Thr	Val	Gly	Ile	Tyr	Gly	Val	Ile	Leu	1	5	10	15
Ile	Thr	Tyr	Leu	Val	Ile	Lys	Leu	Gly	Leu	Ser	Phe	Leu	Tyr	Glu	Pro	20	25	30	
Phe	Lys	Gly	Asn	Pro	His	Asp	Tyr	Lys	Val	Ala	Ala	Val	Ile	Pro	Ser	35	40	45	
Tyr	Asn	Glu	Asp	Ala	Glu	Ser	Leu	Leu	Glu	Thr	Leu	Lys	Ser	Val	Leu	50	55	60	
Ala	Gln	Thr	Tyr	Pro	Leu	Ser	Glu	Ile	Tyr	Ile	Val	Asp	Asp	Gly	Ser	65	70	75	80
Ser	Asn	Thr	Asp	Ala	Ile	Gln	Leu	Ile	Glu	Glu	Tyr	Val	Asn	Arg	Glu	85	90	95	
Val	Asp	Ile	Cys	Arg	Asn	Val	Ile	Val	His	Arg	Ser	Leu	Val	Asn	Lys	100	105	110	
Gly	Lys	Arg	His	Ala	Gln	Ala	Trp	Ala	Phe	Glu	Arg	Ser	Asp	Ala	Asp	115	120	125	
Val	Phe	Leu	Thr	Val	Asp	Ser	Asp	Thr	Tyr	Ile	Tyr	Pro	Asn	Ala	Leu	130	135	140	
Glu	Glu	Leu	Leu	Lys	Ser	Phe	Asn	Asp	Glu	Thr	Val	Tyr	Ala	Ala	Thr	145	150	155	160
Gly	His	Leu	Asn	Ala	Arg	Asn	Arg	Gln	Thr	Asn	Leu	Leu	Thr	Arg	Leu	165	170	175	
Thr	Asp	Ile	Arg	Tyr	Asp	Asn	Ala	Phe	Gly	Val	Glu	Arg	Ala	Ala	Gln	180	185	190	
Ser	Leu	Thr	Gly	Asn	Ile	Leu	Val	Cys	Ser	Gly	Pro	Leu	Ser	Ile	Tyr	195	200	205	
Arg	Arg	Glu	Val	Ile	Ile	Pro	Asn	Leu	Glu	Arg	Tyr	Lys	Asn	Gln	Thr	210	215	220	
Phe	Leu	Gly	Leu	Pro	Val	Ser	Ile	Gly	Asp	Asp	Arg	Cys	Leu	Thr	Asn	225	230	235	240
Tyr	Ala	Ile	Asp	Leu	Gly	Arg	Thr	Val	Tyr	Gln	Ser	Thr	Ala	Arg	Cys	245	250	255	
Asp	Thr	Asp	Val	Pro	Phe	Gln	Leu	Lys	Ser	Tyr	Leu	Lys	Gln	Gln	Asn	260	265	270	
Arg	Trp	Asn	Lys	Ser	Phe	Phe	Arg	Glu	Ser	Ile	Ile	Ser	Val	Lys	Lys	275	280	285	
Ile	Leu	Ser	Asn	Pro	Ile	Val	Ala	Leu	Trp	Thr	Ile	Phe	Glu	Val	Val	290	295	300	
Met	Phe	Met	Met	Leu	Ile	Val	Ala	Ile	Gly	Asn	Leu	Leu	Phe	Asn	Gln	305	310	315	320

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Ala Ile Gln Leu Asp Leu Ile Lys Leu Phe Ala Phe Leu Ser Ile Ile
325 330 335

Phe Ile Val Ala Leu Cys Arg Asn Val His Tyr Met Val Lys His Pro
340 345 350

Ala Ser Phe Leu Leu Ser Pro Leu Tyr Gly Ile Leu His Leu Phe Val
355 360 365

Leu Gln Pro Leu Lys Leu Tyr Ser Leu Cys Thr Ile Lys Asn Thr Glu
370 375 380

Trp Gly Thr Arg Lys Lys Val Thr Ile Phe Lys
385 390 395

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CLAIMS

We claim:

1. An isolated or recombinant nucleic acid which encodes a mammalian hyaluronan synthase.
- 5 2. The nucleic acid of Claim 1 wherein the hyaluronan synthase is human.
3. The nucleic acid of Claim 1 comprising SEQ ID NO: 1.
4. The nucleic acid of Claim 1 wherein said nucleic acid hybridizes under stringent conditions with a second
10 nucleic acid having a nucleotide sequence of SEQ ID NO: 1.
5. The nucleic acid of Claim 1 wherein the nucleic acid encodes the amino acid sequence of SEQ ID NO: 2.
6. A recombinant nucleic acid construct comprising a
15 nucleic acid of Claim 1.
7. The recombinant nucleic acid construct of Claim 6 comprising SEQ ID NO: 1.
8. The recombinant nucleic acid construct of Claim 6 wherein the nucleic acid encodes the amino acid
20 sequence of SEQ ID NO: 2.
9. The recombinant nucleic acid construct of Claim 6 wherein the nucleic acid is operably linked to an expression control sequence.
10. A host cell comprising the nucleic acid of Claim 1.

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11. The host cell of Claim 10 wherein the nucleic acid is operably linked to an expression control sequence, whereby mammalian hyaluronan synthase is expressed when the host cell is maintained under conditions suitable for expression.
12. A method for producing a mammalian hyaluronan synthase comprising:
- a) introducing into a host cell a nucleic acid construct comprising a nucleic acid which encodes a mammalian hyaluronan synthase, whereby a recombinant host cell is produced having said coding sequence operably linked to at least one expression control sequence; and
 - b) maintaining the host cells produced in step a) under conditions whereby the nucleic acid is expressed.
13. An antibody or functional portion thereof which binds mammalian hyaluronan synthase.
14. A method of detecting mammalian hyaluronan synthase in a sample comprising:
- a) contacting a sample with an antibody which binds hyaluronan synthase under conditions suitable for specific binding of said antibody to the mammalian hyaluronan synthase; and
 - b) detecting an antibody-mammalian hyaluronan synthase complex.
15. A method of producing hyaluronan comprising maintaining a host cell of Claim 10 under conditions whereby hyaluronan is produced.

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16. The method of Claim 15, comprising isolating hyaluronan thereby produced.

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FIGURE 1A

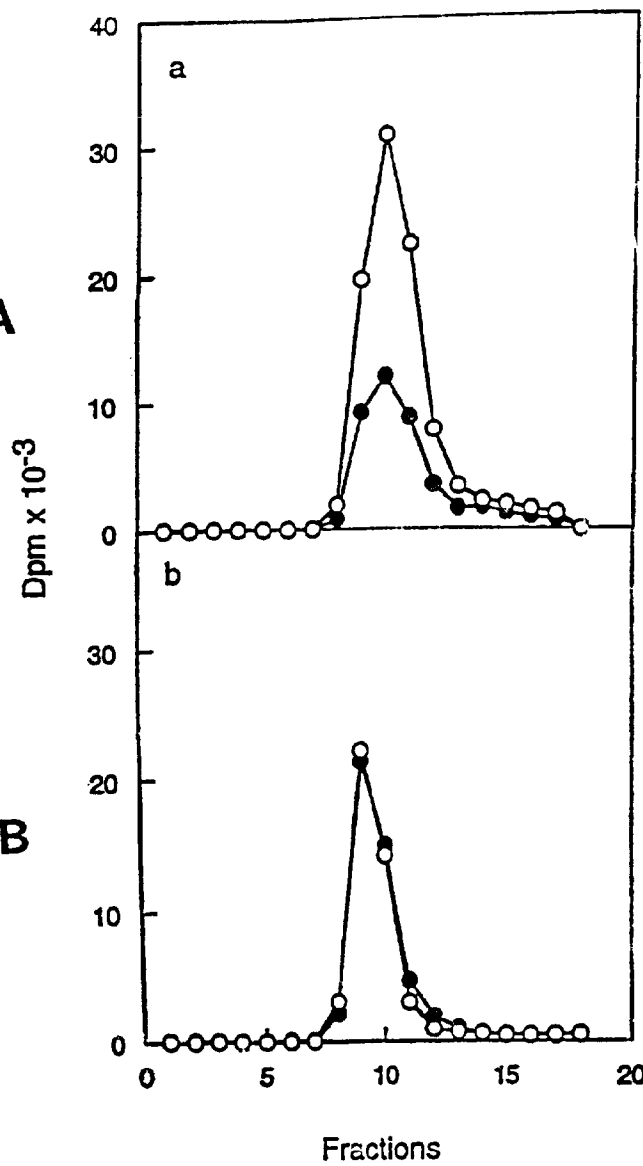


FIGURE 1B

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CCGACAGACAGACAGCCCCGAGACCCACTCCGATGACACAGAGGACGGCCCAAGCCCACTCTCCAGCCCGCCG 80
 H R Q Q D A P K P T P A A R R
 TGCTCCGGCCTGGCCCGGAGGCTGCTGACCATGCGCTTGGCCCTGCTCATCTGGCCCTCATGACCTGGCCCTACGCCG 160
 (C) S G L A R R V L T I A F A L L I L G L M T W A Y A A
 CCGGCTGGCCCTGGCCCTGCGATGCGTACGGCCCTGCTGGCCCTGCGCCCTACCGCCCTTCTTTACGGCCACTGCTGG 240
 G V P L A S D R Y G L L A F G L Y G A F L S A H L Y
 CGCAGAGCCTCTTGGCTACCTGAGCAGCCCGCCGCTGGCCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG 320
 A Q S L F A Y L E H R R Y A A A A R G P L D A A T A R
 AGTGTGGCCCTGACCATCTGCGCTTACCAGGAGGACCCCGCTACCTGCGCCAGTGGCTGGCTGGCCCGCCCGCCCTGCT 400
 S Y A L T I S A Y Q E D P A Y L R Q (C) L A S A (R A L L)
 GTACCCCGCGCGCGCGCTGGCGCTCTCATGGTGGTGGATGGCAACCGCCGAGGACCTCTACATGGTGCACATGTTCC 480
 Y P R A R L R V L H V V D G N R A E D L Y H Y D H F
 CGCAGCTCTTGGCTGACGAGGACCCCGCCAGCTAGCTGTGGGACGGCAACTACCAGCCCTGGGAACCCCGCCCGCGG 560
 R E V F A D E D P A T Y V W G N Y H Q P W E P A A A
 CGCCCGCTGGCCCGCGGAGCCTATCGGAGGTGGAGCGGAGGATCTCGCCCGCTGGCAGTGGAGCGCTGTGAGGAC 640
 G A Y G A G A Y R E V E A E D P G R L A Y E A L V R T
 TCGCAGGTGGCTGTGGCTGGCCAGCGCTGGCCCGCAAGCGGAGGTATGTACACAGCCTTCAAGCGCTGGGAGATT 720
 R R (C) Y (C) V A Q R V G G K R E Y H Y T A F K A L G O
 CGGTGGCTACCTGAGCTGTGTGACTCGGACACAAGGTGGACCCCATGGCACTGCTGGAGCTGCTGGCGCTACTGGAC 800
 S Y D Y V Q Y (C) O S O T R L O P H A L L E L V R Y L O
 GAGCAGCCCGCGCTAGCGCTGTGGTGGGAGCTGGGATCTTAACCTGTGGACTGCTGGCTGAGCTTCTTAAGCAG 880
 E D P R Y G A Y G G D V R I L N P L D S V Y S F L S S
 CCTGCCATACTGGTACCTTCAATGTGGAGCGGCTTGTGAGAGCTACTTCCACTGTGTATCTGCAATCAGCGCTCTC 960
 L R Y V Y A F N Y E R A (C) O S Y F H (C) V S (C) I S G P
 TACGCTATATAGGAATAACCTCTTGCAGCACTTTCTTGAGGCTGGTACAACCAAGCTTCTGGTACCCACTGTACT 1040
 L G L Y R N H L L Q O F L E A V Y N O K F L G T H (C) T
 TTTGGGATGACCGGCACTCAACCGCATGCTGAGCTGGGTATGCTACCAAGTACACCTCCAGTGGCGCTGCTA 1120
 F G Q D (R H L T) M R N L S H G Y A T (K Y T) S R S R (C) Y
 CTCAGAGACGCGCTGCTGCTTGGTGGGCTGAGGAGGACAGACCGGTGGTCCAAGTGTACTTCCGCTAGTGGCTGT 1200
 S E T P S S F L (R V L S) Q O T (R V S) K S Y F R E V L
 ACAAGCGCTCTGCTGGCAGCGGACCATGCTGGATGACCTACGAGCGGCTGGTCTCGCGCTGTTCCGCTTCTTGGT 1280
 Y N A L W V H R N H A V N T Y E A V Y S G L F P F F V
 CGCGGCACTGCTGCTGCTGCTTCTACGCGCGCGCGCTGGCGGCTGCTGTGGTGGTGGTGTGGTGGTGGTGGTGGT 1360
 A A T V L R L F V A G R P V A L L V Y L L (C) Y Q S Y A
 ACTGGCCAAAGCGCGCTTGGCGCGCTGGCTGGCGGCTGGCTGGCATGGTGTTCCTGTGGCTTACGCGCGCTCTACA 1440
 L A K A A F A A V L R G (C) L R H V L L S L Y A P L Y
 TGTGTGGCTTCTGCTGGCAAGTTCCTGGCGCTAGTCAACATGAACCAAGTGGCTGGCGGCACTTGGCGCGCGCGCAAG 1520
 H (C) G L L P A K F L A L Y T H N Q S G W G T S G R R K
 CTGGCGGCTAACTACGTGCTGCTGCTGGCGGCTGGCGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCT 1600
 L A A H Y V P L L P L A L V A L L L L G G L Y R S Y A
 ACACGAGCGCGCGCGCTGAGGCGCGCGCTTCCCGCGCGCGCGCTACCACTTGGCGCGCGCGCGCGCGCGCGCTAGG 1680
 H E A R A D V S G P S R A A E A Y H L A A G A G A Y
 TGGGCTACTGGCTGGCATGTTGACCTGTACTGGTGGCGCTGGCGAGCTTTGGCGCGCGCGCGCGCGCGCGCTACCGG 1760
 Y G Y V Y A H L T L Y W V G Y R R L (C) R R R T G C Y R
 GTCCAGGTGTGAGTCCAGGCAAGCGCTGCTCAAGGCTTTCAGCGGAGGCGAGGAGAGCTGCTGGCGCGCGGAG 1840
 V Q Y
 CCACCAACTTCTGGTGGTGTCTGTGGCGCTGAGTTTCCCTGCTTGGCAAGGAGGGGCTGAGCCCAAGATTCTTCAGT 1920
 CTGCACTATATTGGCACTGGCACTTCTGGGTCTCCAGGAGCGGTATTTATTGGTCAGGATGTCGCAATTTCAGGAGTGGAG 2000
 CCGAAAGGCTCTGCTTCTCTCTCTTATTAACTCCATTCTACTGTGTGATCAGGATGTAATAAACAATTTAT 2080
 TTATTTTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 2116

FIGURE 2

FIGURE 3A

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FIGURE 3B

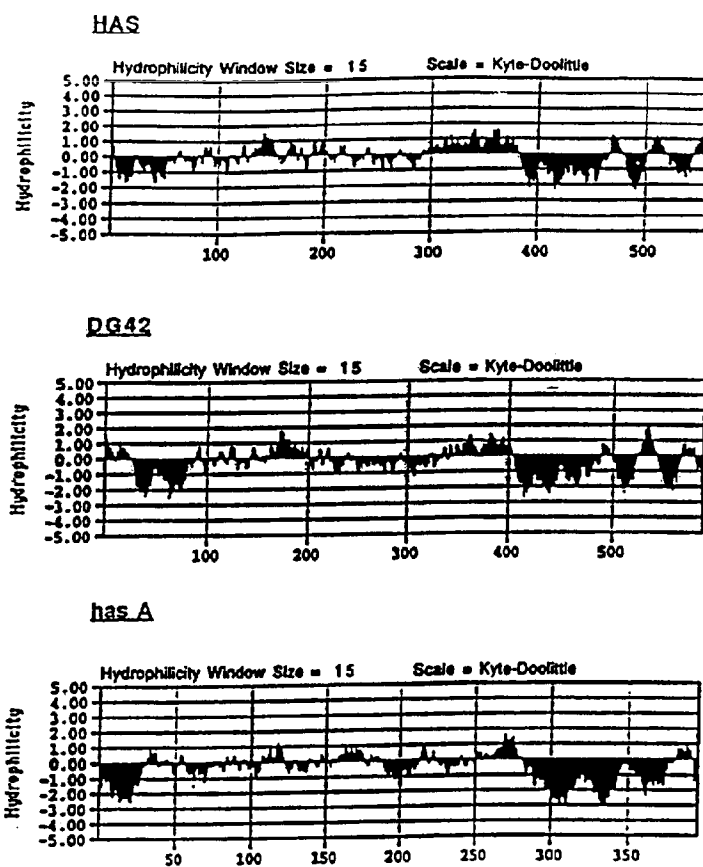
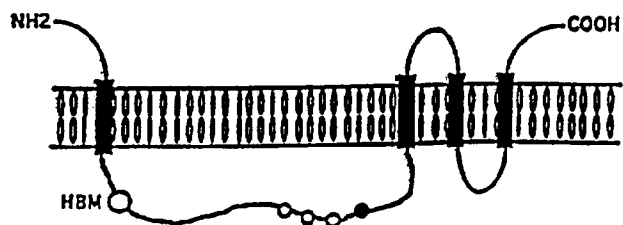


FIGURE 3C



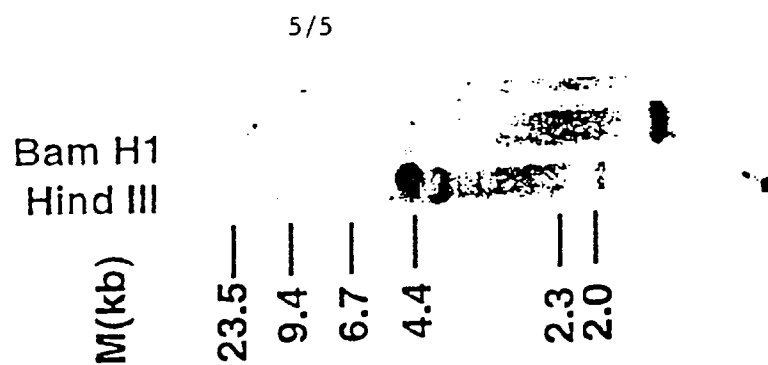


FIGURE 4A

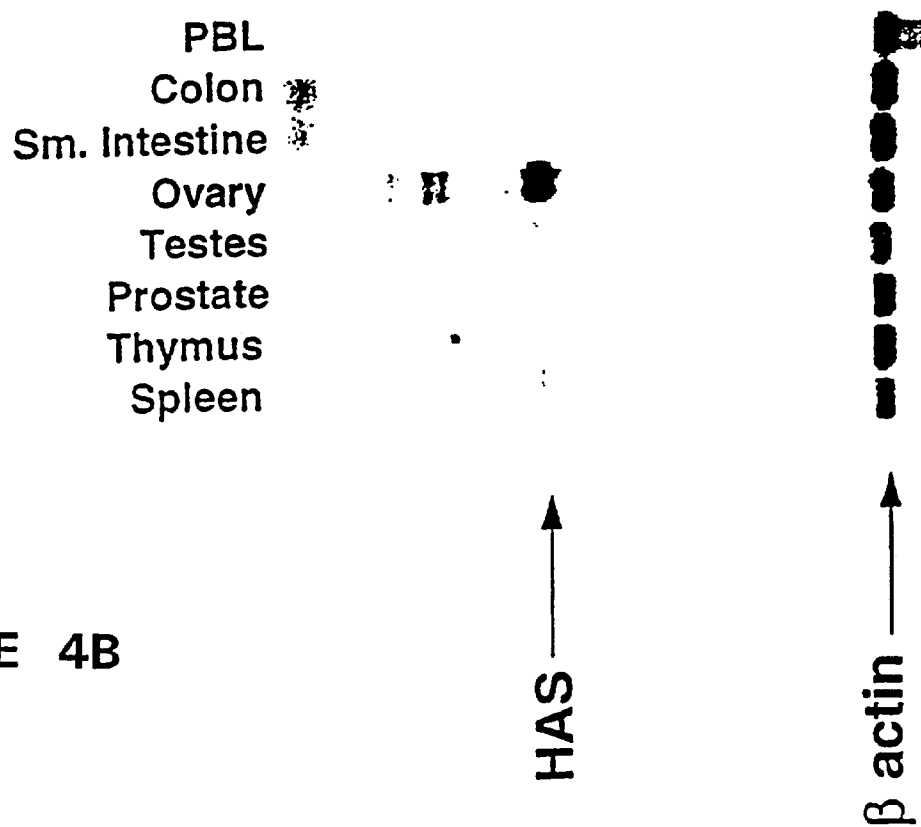


FIGURE 4B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/06350

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/54 C12N5/10 C12N9/10 C07K16/40 G01N33/573
C12P19/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K G01N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>BIOCHEMICAL JOURNAL, vol. 290, no. 3, 15 March 1993, pages 791-795, XP002038783 LUDGER KLEWES ET AL.: "The hyaluronate synthase from a eukaryotic cell line" cited in the application see abstract see page 791, left-hand column, paragraph 1 - right-hand column, paragraph 3 see page 792, right-hand column, paragraph 3 - page 795, right-hand column, paragraph 3</p> <p style="text-align: center;">--- -/--</p>	14

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search

25 August 1997

Date of mailing of the international search report

16.09.97

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 26, 15 September 1993, MD US, pages 19181-19184, XP002038784 PAUL L. DEANGELIS ET AL.: "Molecular cloning, identification, and sequence of the hyaluronan synthase gene from group A Streptococcus pyogenes " cited in the application see the whole document ---	1-16
A	WO 94 00463 A (M.U.R.S.T. ITALIAN MINISTRY FOR UNIVERSITIES) 6 January 1994 see the whole document ---	1-16
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 38, 20 September 1996, MD US, pages 23395-23399, XP002038785 ANNE M. SHYJAN ET AL.: "Functional cloning of the cDNA for a human hyaluronan synthase" see the whole document ---	1-16
P,X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 222, no. 3, 24 May 1996, ORLANDO, FL US, pages 816-820, XP002038786 NAOKI ITANO ET AL.: "Molecular cloning of human hyaluronase synthase" see abstract see page 816, paragraph 2 - paragraph 3; figure 1 see page 818, paragraph 2 - page 820, paragraph 2; figure 2 ---	1-16
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 38, 20 September 1996, MD US, pages 22945-22948, XP002038787 KEN WATANABE ET AL.: "Molecular identification of a putative human hyaluronan synthase" see the whole document ---	1,2,4,6, 9-16
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 14, 4 April 1997, MD US, pages 8957-8961, XP002038788 ANDREW P. SPICER ET AL.: "Molecular cloning and characterization of a cDNA encoding the third putative mammalian hyaluronan synthase" see the whole document ---	1,2,4,6, 9-16

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/06350

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 38, 20 September 1996, MD US, pages 23400-23406, XP002038789 ANDREW P. SPICER ET AL.: "Molecular cloning and characterization of a putative mouse hyaluronan synthase" see abstract see page 23400, right-hand column, paragraph 2; figure 2 see page 23402, right-hand column, paragraph 2 - page 23406, left-hand column, paragraph 1</p> <p style="text-align: center;">---</p>	1,6,9-16
P,X	<p>JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 17, 26 April 1996, MD US, pages 9875-9878, XP002038790 NAOKI ITANO ET AL.: "Expression cloning and molecular characterization of HAS protein, a eukaryotic hyaluronan synthase" see abstract see page 9877, left-hand column, paragraph 2 - page 9878, right-hand column, paragraph 2; figure 1</p> <p style="text-align: center;">---</p>	1,6,9-16
P,X	<p>ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, vol. 337, no. 2, 15 January 1997, pages 261-266, XP002038791 CSABA FÜLOP ET AL.: "Coding sequence of a hyaluronan synthase homologue expressed during expansion of the mouse cumulus-oocyte complex" see abstract see page 261, right-hand column, paragraph 3 see page 262, left-hand column, last paragraph - right-hand column, paragraph 3; figure 1</p> <p style="text-align: center;">-----</p>	1,6,9-16

information on patent family members

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